



Evaluation of Antitumor Activity of Novel 1,2,4 Triazole Derivatives: An *in vitro* and *in vivo* Model

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ABSTRACT

Cancer is one of the leading causes of death worldwide in spite of the immense efforts in the search for effective anti-cancer drugs. Triazoles are the class of heterocyclic compounds which has a wide variety of activity, low toxicity and good Pharmacokinetic and Pharmacodynamics profiles. The present study aims to investigate the anti-tumor activity of the novel 1,2,4 triazole derivatives (viz., MPA and OBC) which were synthesized in Dept. of Chemistry laboratory, JSS College of Pharmacy, Mysore. The compounds were subjected for *in vitro* cytotoxicity studies like – Sulforhodamine B assay and DNA fragmentation assay further the compounds were evaluated for anti-tumor activity against Ehrlich Ascite Carcinoma (EAC) and Dalton Lymphoma Ascites (DLA) induced tumours in Swiss Albino Mice. In EAC model, anti-tumor activity was evaluated by change in body weight, mean survival time and hematological parameters. In DLA model, tumor volume and tumor weight was evaluated. Acute toxicity studies of MPA and OBC were done according to OECD – 423 guidelines and were found to be safe at 300 mg/kg. Both the compounds showed good cytotoxic potential by *in-vitro* cytotoxicity assays. The novel 1,2,4 triazole derivatives was found to possess the antitumor activity on liquid and solid tumor model which is very much nearer to the standard group. However, further detail research is required to establish anti-tumor activity of novel 1,2,4 triazole derivatives.

Keywords: 1,2,4 triazole derivatives; *in vitro*; *in vivo*; EAC; DLA

INTRODUCTION

Cancer is the second leading cause of death globally and accounted for 8.8 million deaths in 2015. Many cancers can be prevented by avoiding exposure to common risk factors, such as tobacco smoke, UV radiation, chemicals, etc. In addition, a significant proportion of cancers can be cured, by surgery, radiotherapy or chemotherapy, especially if they are detected early. To reduce the significant disability, suffering and deaths caused by cancer worldwide, effective and affordable programmes in early diagnosis, screening, treatment, and palliative care are needed [1]. A promising strategy for cancer prevention today is chemotherapy [2].

The discovery of cancer drug continues to develop at a phenomenal pace and huge amounts of alternatives are engaged for drug discovery and design [3]. In spite of the massive efforts in the search for effective anti-cancer drugs, it is one of the leading causes of death in the world while the long-term prediction is still unfavourable [4]. A recent report suggests around 14.1 million new cases and 8.2 million cancer-related deaths, which are expected to reach 21.7 million and 13 million by 2030 worldwide, respectively [5].

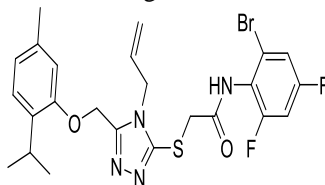
The substances containing a five member heterocyclic base are significant targets in chemical synthesis because of their prominent biological activities [6]. 1,2,4-Triazole derivatives are acknowledged to exhibit a broad series of biological activities, such as antibacterial [7-9], antifungal [10], analgesic [11], antitubercular [12], antidepressant [13], anti-inflammatory [14], antiviral [15], central nervous system (CNS) [16], anticonvulsant [17], hypoglycaemic [18], antimalaria [19], antiplatelet [20], antioxidant [21] and antitumor [22-24]. 1,2,4-Triazole find relevance in the management of a number of diseases [25].

There are also some existing drugs containing 1, 2, 4-triazole moiety, e.g. Triazolam, Alprazolam, Etizolam, Furacylin, Ribavirin, Hexaconazole, Triadimefon, Mycobutanil Rizatriptan, Propiconazole, Fluotrimazole which are extensively used for various disorders [26]. In the present study, two recently developed triazole derivatives – 2-Phenoxy alkyl-4-phenyl-5-substituted mercapto 1,2,4-triazole (MPA and OBC.) were taken for the study for anticancer activity.

EXPERIMENTAL SECTION

Compounds

Two 1,2,4-triazole derivatives were obtained as samples from Dr. Madhusudan N Purohit, Professor, Dept. of Pharmaceutical Chemistry, JSS College of Pharmacy, Mysore. Recently developed triazole derivatives – 2-Phenoxy alkyl-4-phenyl-5-substituted mercapto 1,2,4-triazole (MPA and OBC code name given resp.) were taken for the study for anticancer activity. The compounds were weighed and stored in an air-tight container at room temperature.



MMs02943764 (Parent molecule)

General chemical name

2-Phenoxy alkyl 4-phenyl-5-substituted mercapto 1,2,4-triazole

Animals

The experiment was carried out on male Swiss Albino Mice weighing between 25-35 gm which are procured from In-vivo Biosciences (Reg. no. 971/bc/06/CPCSEA), Bangalore. The animal care and handling was carried out in accordance to CPCSEA guidelines issued by the Institutional Animal Ethics Committee, JSS College of Pharmacy, Mysore, Karnataka. Animals were acclimatized to the experimental condition for one week prior to the experiment. Animals were maintained under controlled conditions of temperature ($27 \pm 2^\circ\text{C}$) and were caged in sterile polypropylene cages containing sterile paddy husk as bedding material with maximum of six animals in each cage. The mice were fed on standard food pellets and water ad libitum. The studies conducted were approved by the Institutional Ethical Committee, JSS College of Pharmacy, Mysore, Karnataka (Approval no: P8 238/2017).

***In vitro* Anticancer Evaluation against MDA MB – 468 Breast Cancer Cell Lines Using Sulforhodamine B Assay**

Sulforhodamine B assay is based on the ability of SRB to bind electrostatically to the protein components of the cells and pH dependent on protein basic amino acid residues of trichloroacetic acid (TCA) fixed cells. SRB is an anionic bright pink aminoxanthene protein dye with two sulfonic groups. The amount of dye extracted from stained cells is directly proportional to the cell mass [27,28]. The cell lines at 5×10^3 cells/well were plated in 96 well plates 24 h before treatment with the compounds to allow attachment of cell to the wall of the plate. Different concentrations of the compounds under test (31.25, 62.5, 125, 250, 500, 1000, 2000 μM) were added to the cell monolayer and incubated for 24 h and 48 h. The assay was terminated after the completion of specified incubation period. The % cytotoxicity of the compounds was determined for all the duration and IC₅₀ was determined. The activity was done in triplicate and results are expressed as Mean \pm SEM.

DNA Fragmentation Assay

A distinctive biochemical feature of apoptosis is the fragmentation of DNA by a specific nuclease called caspase-activated DNase (CAD). Activation of CAD by the caspase cascade leads to specific cleavage of DNA at

internucleosomal linker sites, generating fragments of ~200 base pairs known as DNA ladders [29]. The cell lines were plated in 6 well plates and the compounds were given at three different concentrations (1000, 2000 and 3000 μ M). The DNA was then precipitated and loaded in agarose gel [30].

Acute Toxicity Studies: Determination of Maximum Tolerable Dose (MTD) of Selected Compound by OECD-423 Guidelines

Maximum tolerable dose (MTD) is the highest dose of a chemical or substance that an animal species can tolerate without significant impairment or adverse effects other than carcinogenicity. The protocol used to carry out the acute toxicity study in mice was as per OECD – 423 guidelines [31]. The doses selected were based on the dose progression factor. A stock drug concentration of 300 mg/kg was prepared in 0.5% CMC just prior to administration and suitably diluted to get the required drug concentrations.

Ehrlich Ascites Carcinoma (EAC) Induced Liquid Tumor Model

EAC cells were obtained from the peritoneal cavity of an EAC bearing mouse, after 15 days of tumor transplantation. The ascetic fluid was drawn intraperitoneally using a sterile syringe and a small amount was tested for microbial contamination. EAC cells were aspirated from peritoneal cavity of the tumor bearing mice and 0.1 ml of 10⁶ cells/ml was injected intraperitoneally into the Swiss albino mice [32-33]. Treatment was started after 24 h tumor inoculation and continued for 15 days daily dosing regimen. The anti-tumour activity was assessed by morphological, haematological and % increase in lifespan (%ILS).

Treatment Schedule

42 Swiss albino mice were used in the experiment, which were divided into seven groups (n=6), they were fed with food and water ad libitum. All the animals in each groups received EAC Cells except Group-I. This was taken as day '0'. Group-I animals served as normal Sodium CMC (0.5%) p.o. for 15 days and group-II animals served as control where cells are inoculated without any treatment. 24 h after EAC transplantation, Group-III animals received reference drug cisplatin (3.5 mg/kg i.p) on two alternate days regimen for 15 days. Groups-IV and V animals received MPA at a dose of 25 and 50 mg/kg orally and groups-VI and VII animals received OBC at a dose of 25 and 50 mg/kg orally for 15 days respectively. After 24 hours of the last dose and then 18 hour of fasting, blood were collected from retro-orbital plexus of the animals and kept them for survival study for another 15 days.

Dalton's Lymphoma Ascites (DLA) Induced Solid Tumor Model

The tumor cells (DLA Cells) were aspirated from the peritoneal cavity of tumor bearing mice, after 15 days of tumor transplantation. The ascitic fluid was drawn using an 18-gauge needle into a sterile syringe and a small amount was tested for microbial contamination. DLA cells were aspirated from peritoneal cavity of the tumor bearing mice and 0.1 ml of 10⁶ cells/ml was injected intramuscularly into the right hind limb of Swiss albino mice [34]. The solid tumor then developed in around 12 days; treatment was started after 24 h tumor inoculation and continued for 15 days by alternate dosing regimen.

Treatment Schedule

30 Swiss albino mice were used in the experiment, which were divided into five groups (n=6), they were fed with food and water ad libitum. All the animals in each groups received DLA Cells. This was taken as day '0'. Group-I animals served as control where cells are inoculated without any treatment. 24 h after DLA transplantation, Group-II animals received reference drug cisplatin (3.5 mg/kg i.p) on two alternate days regimen for 15 days. Groups-III, IV and V animals received OBC at a dose of 25, 50 and 100 mg/kg orally for 15 alternate days respectively. After 24 hours of the last dose and then 18 hour of fasting, animals of each group were sacrificed by cervical dislocation to measure tumor volume and tumor weight.

RESULTS AND DISCUSSION

Before commencing the experiments of cytotoxicity, preliminary screening of compounds MPA and OBC in media was determined in MDA MB-468 cell line by SRB assay. The percentage cytotoxicity was assessed at different time points, i.e. (24 h and 48 h). The result showed that the compound OBC was more effective than MPA in showing cytotoxicity at 48 h exposure (Tables 1-4).

Table 1: Cytotoxicity study of novel 1,2,4 triazole derivatives by SRB assay using MDA MB – 468 cell line (24 h)

Sl. No.	Compounds	% Cytotoxicity after 24 h Exposure at 500 μ M and 2000 μ M on MDA MB – 468 Cell Line	
		500 μ M	2000 μ M
1	MPA	40.7	56.3
2	OBC	41	57.8

Table 2: Cytotoxicity study of novel 1,2,4 triazole derivatives by SRB assay using MDA MB – 468 cell line (48 h)

Sl. No.	Compounds	% Cytotoxicity after 48 h Exposure at 500 μ M and 2000 μ M on MDA MB – 468 Cell Line	
		500 μ M	2000 μ M
1	MPA	30.6	56
2	OBC	34.3	60.3

In vitro cytotoxicity of novel 1,2,4 triazole derivatives on MDA MB – 468 cell lines and determination of IC₅₀ by SRB assay.

Table 3: % Cytotoxicity study of novel 1,2,4 triazole derivatives by SRB assay using MDA MB – 468 cell line (24 h)

Compounds	% Cytotoxicity after 24 h Exposure By Seven Dose Assay						
	31.25 μ M	62.5 μ M	125 μ M	250 μ M	500 μ M	1000 μ M	2000 μ M
MPA	21.80 \pm 0.40	25.26 \pm 1.16	27.69 \pm 0.81	38.31 \pm 0.08	41.72 \pm 1.20	43.11 \pm 0.70	58.63 \pm 0.83
OBC	17.60 \pm 0.80	25.40 \pm 1.20	33.65 \pm 1.25	39.00 \pm 1.00	42.30 \pm 1.29	47.65 \pm 0.35	57.25 \pm 0.95

All values are expressed as mean \pm SEM, n=3. % inhibition was calculated by plotting % inhibition vs. concentration using non-linear regression

Table 4: % Cytotoxicity study of novel 1,2,4 triazole derivatives by SRB assay using MDA MB – 468 cell line (48 h)

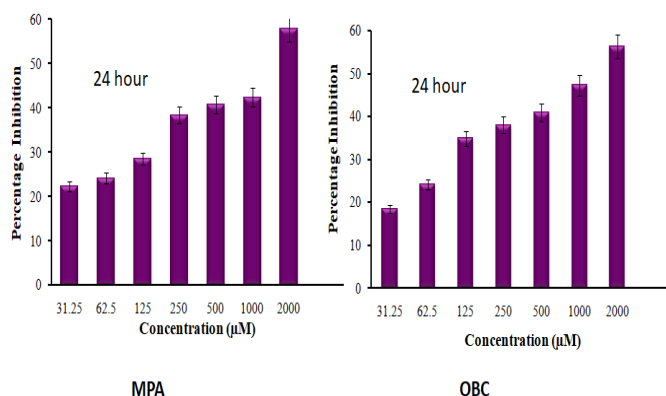
Compounds	% Cytotoxicity after 48 H Exposure By Seven Dose Assay						
	31.25 μ M	62.5 μ M	125 μ M	250 μ M	500 μ M	1000 μ M	2000 μ M
MPA	7.95 \pm 0.55	14.93 \pm 0.43	15.94 \pm 0.54	24.17 \pm 1.37	31.55 \pm 0.94	41.06 \pm 0.83	57.05 \pm 1.04
OBC	9.65 \pm 1.15	12.35 \pm 1.05	17.15 \pm 0.35	23.70 \pm 1.20	33.65 \pm 0.64	44.35 \pm 1.15	59.65 \pm 0.64

All values are expressed as mean \pm SEM, n=3. % inhibition was calculated by plotting % inhibition vs. concentration using non-linear regression

Based on the percentage cytotoxicity obtained from the preliminary screening of both the compounds, both MPA and OBC were subjected to detailed in-vitro study using SRB assay by seven doses, for finding maximum

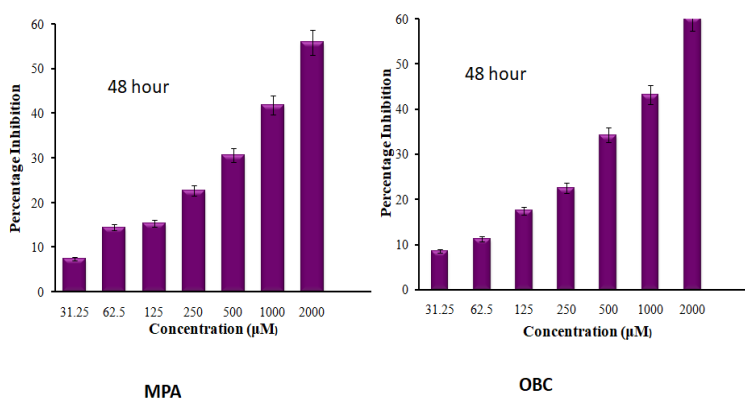
percentage cytotoxicity and IC50. The maximum cytotoxicity was observed by novel compound OBC at the dose of 2000 μM with the percentage of cytotoxicity (59.65%) and IC50 value was found to be 1281 μM after 48 h. MPA also showed good effect on 24 h as the IC50 is less compared to 48 h. After 48 hrs the cytotoxicity was increased more compared with 24 h in dose dependant manner. It shows the stability of the compounds and long term cytotoxic potential (Graph 1 and Graph 2).

Graph 1: % Cytotoxicity study of novel 1,2,4 triazole derivatives by SRB assay using MDA MB – 468 cell line at 24 h



values are expressed as mean \pm SEM, n=3. % inhibition was calculated by plotting % inhibition vs. concentration using non-linear regression.

Graph 2: % Cytotoxicity study of novel 1,2,4 triazole derivatives by SRB assay using MDA MB.



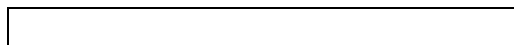
All values are expressed as mean \pm SEM, n=3. % inhibition was calculated by plotting % inhibition vs. concentration using non-linear regression.

The IC50 value of MPA was 1202 μM and 1522 μM at 24 h and 48 h respectively. The IC50 value of OBC was 1009 μM and 1281 μM at 24 h and 48 h respectively. It shows that OBC has lower IC50 value than MPA (Table 5).

Table 5: IC50 values of novel 1,2,4 triazole derivatives by SRB assay using MDA MB – 468 cell line at 24 and 48 h

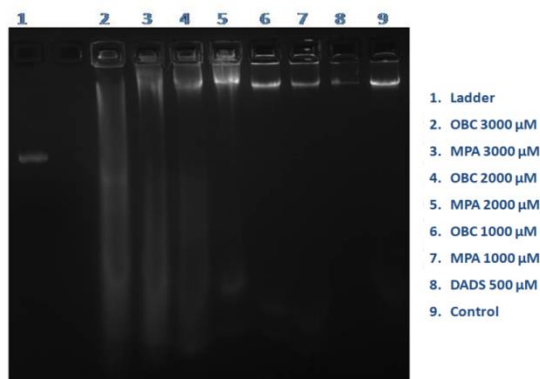
Sl. No	Compounds	24 h	48 h
1	MPA	1202 μM	1522 μM
2	OBC	1009 μM	1281 μM

Inhibitory concentration 50 (IC50) was calculated by plotting % cytotoxicity vs. concentration using non-linear regression



DNA Fragmentation Assay

Figure 1: Agarose gel electrophoresis showing nucleosomal DNA fragmentation of MDA MB – 468 cells induced by novel 1,2,4 triazole derivatives



Gel electrophoresis assay of DNA from MDA MB-468 cell lines were treated with MPA and OBC at different concentration of 1, 2 and 3 mM (1000, 2000, 3000 μ M) for 48 h. 2000 and 3000 μ M showed good fragmentation in the form of streaking observed in the band which indicates cell death by apoptosis whereas the lower concentration (1000 μ M) didn't show band but slight streaking was observed (Figure 1). MDA MB-468 cells treated with test compound MPA and OBC demonstrated DNA fragmentation as a result of DNA damage when compared to control. However, OBC compound was effective in inducing apoptosis as indicated by DNA fragmentation.

Acute Toxicity Study-Maximum Tolerance Dose

The maximum tolerance dose of MPA and OBC was found to be 300 mg/kg b.w. according to OECD guideline – 423. So, in the *in vivo* study, the animals were treated with the compounds at a dose of 25 and 50 mg/kg. Compound OBC was tested at 2000 mg/kg b.w. and was found to be safe. For the DLA model, 25, 50 and 100 mg/kg b.w. of compound OBC was chosen.

EAC Model

% increase in body weight

Table 6: Antitumor activity of novel 1,2,4 triazole derivatives on EAC induced liquid tumor model (% increase in body weight)

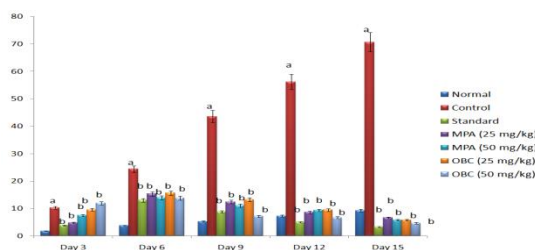
Group	Day 3	Day 6	Day 9	Day 12	Day 15
Normal	1.74 \pm 0.62	3.69 \pm 0.60	5.24 \pm 0.58	7.19 \pm 0.60	9.26 \pm 0.61
Control	10.26 \pm 1.34 ^a	24.35 \pm 1.28 ^a	43.58 \pm 1.37 ^a	56.20 \pm 1.52 ^a	70.71 \pm 1.90 ^a
Standard (Cisplatin 3.5 mg/kg)	3.83 \pm 0.63 ^b	13.06 \pm 1.12 ^b	8.82 \pm 0.77 ^b	4.98 \pm 0.65 ^b	3.24 \pm 0.58 ^b
MPA (25 mg/kg)	4.74 \pm 0.67 ^b	15.34 \pm 0.57 ^b	12.39 \pm 0.25 ^b	8.64 \pm 0.33 ^b	6.59 \pm 0.30 ^b
MPA (50 mg/kg)	7.45 \pm 1.08 ^b	13.97 \pm 1.57 ^b	11.03 \pm 1.74 ^b	9.33 \pm 1.45 ^b	5.75 \pm 1.30 ^b
OBC (25 mg/kg)	9.55 \pm 0.45 ^b	15.64 \pm 0.73 ^b	13.200.61 ^b	9.42 \pm 0.52 ^b	5.76 \pm 0.54 ^b
OBC 50 mg/kg)	11.95 \pm 0.72 ^b	13.78 \pm 1.48 ^b	7.18 \pm 1.44 ^b	6.64 \pm 1.42 ^b	4.61 \pm 1.34 ^b

Values are Mean \pm SEM, n=6, Statistical analysis-One way ANOVA followed by Turkey's test multiple comparisons; a – (P<0.05)

compared with normal group; b – (P<0.05) compared with control group

EAC cells were injected into the peritoneal cavity of the mice to obtain the liquid tumor in a concentration of 106 cells/ml. EAC cells were aspirated from the peritoneal cavity of tumor bearing mice, after 15 days of tumor transplantation. This tumor grows in the peritoneal cavity of the mice in around 12-14 days after tumor inoculation. MPA and OBC at 25 and 50 mg/kg were tested on EAC liquid tumor model in mice. The maximum gain in body weight was observed in control (70.71%) and minimum gain in body weight was seen in cisplatin treated group (3.24%). The novel compounds MPA and OBC showed cytotoxicity in dose dependant manner. At 25 and 50 mg/kg dose, MPA showed 6.59% and 5.75% increase in body weight respectively and OBC showed 5.76% and 4.61% increase in body weight respectively. Due to its cytotoxic property, it reduced the more inflammation in peritoneal cavity further the infiltration of proliferative cells got decreased in turn it reduced the body weight (Graph 3). The maximum body weight reduction was seen at OBC at the dose of 50 mg/kg (Table 6).

Graph 3: Antitumor activity of novel 1,2,4 triazole derivatives on EAC induced liquid tumor model (% increase in body weight)



Values are Mean ± SEM, n=6, Statistical analysis-One way ANOVA followed by Turkey’s test multiple comparisons; a – (P<0.05) compared with normal group; b – (P<0.05) compared with control group.

% increase in life span

After 15th day of treatment the life span assessment was started and cisplatin treated group shown 88.88% increase in life span compared with the control. MPA 25 and 50 mg/kg has 21.11% and 38.88% increase in life span in mice respectively whereas, OBC 25 and 50 mg/kg showed 38.88% and 50% increase in life span (Table 7 and Graph 4).

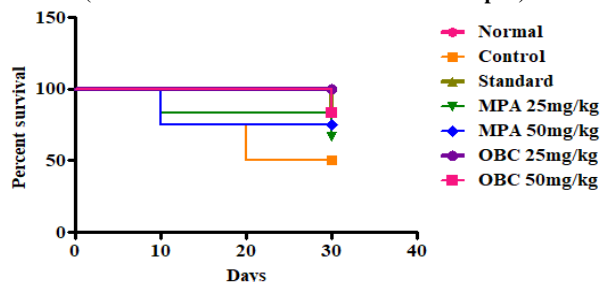
Table 7: Antitumor activity of novel 1,2,4 triazole derivatives on EAC induced liquid tumor model (Mean survival time and % increase in life span)

Group	Mean Survival Time (in days)	% Increase in Life Span
Control	16.16 ± 0.44	-
Standard	28.33 ± 0.84 ^a	88.88
MPA (25 mg/kg)	18.16 ± 0.60 ^{a,b}	21.11
MPA (50 mg/kg)	20.83 ± 0.70 ^{a,b}	38.88
OBC (25 mg/kg)	20.83 ± 0.47 ^{a,b}	38.88
OBC (50 mg/kg)	22.50 ± 0.92 ^{a,b,c}	50

Values are Mean ± SEM, n=6, Statistical analysis-One way ANOVA followed by Turkey’s test multiple comparisons; a – (P<0.05) compared with control group; b – (P<0.05) compared with standard group (cisplatin)

3.5 mg/kg), c – (P<0.05)
compared with MPA 25 mg/kg

Graph 4: Antitumor activity of novel 1,2,4 triazole derivatives on EAC induced liquid tumor model by Log-rank (Mantel-Cox) Test (Mean survival time and % increase in life span)



Haematological Parameters

In haematological parameters in EAC induced mice, more amount of WBC cells and less amount of RBC and Hb were observed. Because of more inflammatory mediators, certain cancer and immune disorder, there was more proliferation of WBC cells in the body. In control animal, higher amount of WBC cell count was observed and lesser RBC count and Hb content. Due to the haemolysis or myelosuppression, RBC synthesis in the body got decreased and more amounts of immature RBC cells were present in the blood. This in turn leads to the low Hb content. The cisplatin treated group showed alteration in the WBC cell count with% decrease of 61.33% compared with control. MPA 25 and 50 mg/kg showed % decrease of 35.72% and 39.37% resp. and OBC 25 and 50 mg/kg showed % decrease of 38.74% and 44.78% resp. when compared to control. Improvement in RBC cell count has been observed. Improvement in Hb content has also been observed with % increase of 85.77% in standard when compared to control. MPA 25 and 50 mg/kg showed % increase of 19.77% and 45.77% resp. and OBC 25 and 50 mg/kg showed % increase of 39.11% and 56.88% resp. when compared to control. All 25 and 50 mg/kg doses showed dose dependant activity to modify haematological parameters compared with the cisplatin group (Graph 5 and Table 8).

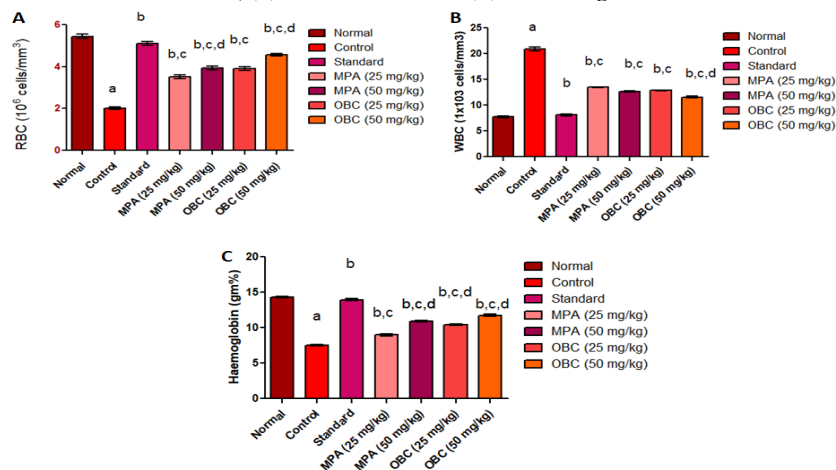
Table 8: Antitumor activity of novel 1,2,4 triazole derivatives on EAC induced liquid tumor model (haematological parameters)

Treatment	RBC	WBC	Haemoglobin
	(1 × 10 ⁶ cells/mm ³)	(1 × 10 ³ cells/mm ³)	(gm%)
Normal	5.45 ± 0.11	7.71 ± 0.19	14.30 ± 0.12
Control	2.01 ± 0.07 ^a	20.95 ± 0.37 ^a	7.50 ± 0.11 ^a
Standard	5.10 ± 0.08 ^b	8.10 ± 0.19 ^b	13.93 ± 0.16 ^b
MPA (25 mg/kg)	3.51 ± 0.08 ^{b,c}	13.46 ± 0.09 ^{b,c}	8.98 ± 0.13 ^{b,c}
MPA (50 mg/kg)	3.93 ± 0.09 ^{b,c,d}	12.70 ± 0.12 ^{b,c}	10.43 ± 0.09 ^{b,c,d}
OBC (25 mg/kg)	3.90 ± 0.08 ^{b,c}	12.83 ± 0.09 ^{b,c}	10.93 ± 0.09 ^{b,c,d}
OBC (50 mg/kg)	4.56 ± 0.06 ^{b,c,d}	11.56 ± 0.16 ^{b,c,d}	11.77 ± 0.12 ^{b,c,d}

Values are Mean ± SEM, n=6, Statistical analysis-One way ANOVA followed by Turkey's test multiple comparisons; a – (P<0.05) compared with normal group; b – (P<0.05) compared with negative control group; c-(P<0.05) compared with Standard Cisplatin (3.5 mg/kg) treated group; d-(P<0.05)

compared with MPA 25 mg/kg group

Graph 5: Antitumor activity of novel 1,2,4 triazole derivatives on EAC induced liquid tumor model (haematological parameters) (A) on RBC Count, (B) on WBC Count and (C) on Haemoglobin count



Values are Mean ± SEM, n=6, Statistical analysis-One way ANOVA followed by Turkey’s test multiple comparisons; a – (P<0.05) compared with normal group; b – (P<0.05) compared with negative control group; c- (P<0.05) compared with Standard Cisplatin (3.5 mg/kg) treated group; d-(P<0.05) compared with MPA 25 mg/kg group.

DLA Model

DLA cells were injected into the hind limb of the mice to obtain the solid tumor in concentration of 106 cells/ml. DLA cells were aspirated from the peritoneal cavity of tumor bearing mice, after 15 days of tumor transplantation. This tumor grows on the limbs of the mice in around 12-14 days after tumor inoculation. OBC at 25, 50 and 100 mg/kg were tested on DLA solid tumor model in mice. In control animals we observed more tumor volume and increased in tumor weight and cisplatin treated group showed more reduction in tumour volume and tumor weight (Graphs 6 and 7). The 100 mg/kg dose shown more similar effect on tumor volume and tumour weight reduction compared with cisplatin treated group (Tables 9 and 10).

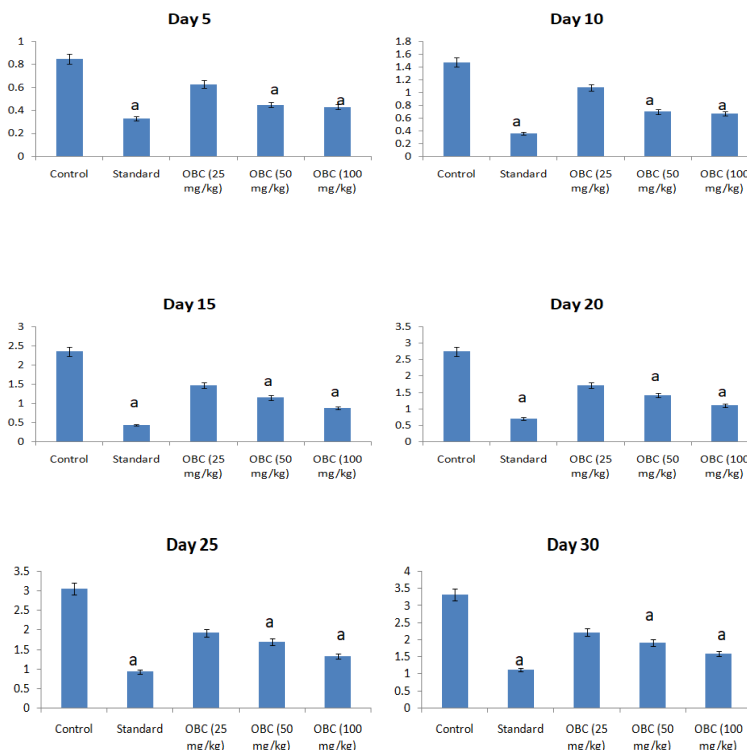
Tumor Volume

Table 9: Antitumor activity of novel 1,2,4 triazole derivatives on DLA induced solid tumor model (tumor volume)

Groups	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Control	0.85 ± 0.04	1.48 ± 0.10	2.36 ± 0.08	2.75 ± 0.09	3.06 ± 0.07	3.33 ± 0.08
Standard (Cisplatin 3.5 mg/kg)	0.33 ± 0.03 ^a	0.36 ± 0.36 ^a	0.43 ± 0.04 ^a	0.70 ± 0.05 ^a	0.93 ± 0.04 ^a	1.12 ± 0.04 ^a
OBC (25 mg/kg)	0.63 ± 0.03	1.08 ± 0.06	1.48 ± 0.04	1.72 ± 0.05	1.93 ± 0.04	2.22 ± 0.04
OBC (50 mg/kg)	0.45 ± 0.04 ^a	0.70 ± 0.05 ^a	1.15 ± 0.04 ^a	1.42 ± 0.04 ^a	1.70 ± 0.03 ^a	1.92 ± 0.03 ^a
OBC (100 mg/kg)	0.43 ± 0.02 ^a	0.67 ± 0.04 ^a	0.88 ± 0.05 ^a	1.10 ± 0.05 ^a	1.33 ± 0.08 ^a	1.60 ± 0.0 ^a

Values are Mean ± SEM, n=6, Statistical analysis-One way ANOVA followed by Turkey’s test multiple comparisons; a – (P<0.05) compared with control group; b – (P<0.05) compared with standard group (cisplatin 3.5 mg/kg)

Graph 6:-Antitumor activity of novel 1,2,4 triazole derivatives on DLA induced solid tumor model (tumor volume)



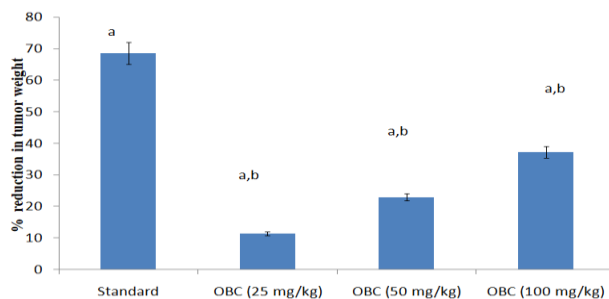
Values are Mean ± SEM, n=6, Statistical analysis-One way ANOVA followed by Turkey’s test multiple comparisons; a – (P<0.05) compared with control group; b – (P<0.05) compared with standard group (cisplatin 3.5 mg/kg).

Tumor Weight

Table 10: Antitumor activity of novel 1,2,4 triazole derivatives on DLA induced solid tumor model (tumor weight and % reduction in tumor weight)

Groups	Tumor weight (% reduction in tumor weight)
Control	8.61 ± 0.13
Standard	2.70 ± 0.23 (68.66) ^a
OBC (25 mg/kg)	7.63 ± 0.14 (11.41) ^{a,b}
OBC (50 mg/kg)	6.63 ± 0.19 (23.01) ^{a,b}
OBC (100 mg/kg)	5.40 ± 0.10 (37.33) ^{a,b}

Values are Mean ± SEM, n=6, Statistical analysis-One way ANOVA followed by Turkey’s test multiple comparisons; a – (P<0.05) compared with control group; b – (P<0.05) compared with standard group (cisplatin 3.5 mg/kg).

Graph 7: Antitumor activity of novel 1,2,4 triazole derivatives on DLA induced solid tumor model (tumor weight and % reduction in tumor weight)

Values are Mean \pm SEM, n=6, Statistical analysis-One way ANOVA followed by Turkey's test multiple comparisons; a – (P<0.05) compared with control group; b – (P<0.05) compared with standard group (cisplatin 3.5 mg/kg).

CONCLUSION

The present study was focused on evaluating newly synthesized novel 1,2,4 triazole derivatives for their antitumor activity by both *in vitro* and *in vivo* studies. The antitumor activity was shown which was obvious from *in vitro* cytotoxicity assay on MDA MB-468 breast cancer cell line. The *in vitro* results were well supported by *in vivo* model assessed against the selected compounds. Thus, the study is an initial step in identification of a novel antitumor agent in spite of many adverse effects of anticancer chemotherapy. However, further detailed investigations are necessary to investigate the mechanism of action of this novel molecule which may bring promising results in cancer chemotherapy.

Future Perspective of the Study

- Sensitivity of compounds on different cancer cell lines.
- Effect of compounds on DNA damage by Comet assay
- Effect of compounds on cell cycle analysis.
- Illustrating the detailed mechanism of action in the promising compounds.

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